CARDIAC MUSCLE CELLS IN MAN AND CERTAIN OTHER MAMMALS.

BY BORDMAN L. OVIATT, B. S., Cornell University, Ithaca, N. Y.

Historical Sketch.

" Leuwenhoek and after him Kölliker," says Rauvier, "demonstrated first that the muscular substance of the heart (or myocardium) is composed of primary fibers analagous to those of ordinary striated muscle, pale and red, but differing in this that they form a multitude of branchings of a Y shape, and anastomose among themselves in such a way as to form a vast contractile network" (4,296).* Kölliker recognized the cardiac cell as the histological element of the heart, but he also held that the cells were merely fused with one another, and not cemented together (3,579). He used, as an argument for this, the fact that in the same segment, isolated by the action of potassium hydrate (M. 1), * one often finds two nuclei.

Ranvier in his (4.296) *Lesons D'Anatomie General*, in opposing this opinion, says: "You understand, gentlemen, that this argument is of no value. We know that many elimentary cells are multinucleated. But the existence, besides, of lines of cement in the structure of the branching cardiac fibers, which may be demonstrated by careful work, renders the opinion of Köllicker entirely untenable." Ranvier adds (5.541), as an explanation for the presence of the two nuclei, the following: "It is more probable that the existence of

* The first numeral refers to the number of the author's work in the bibliography, the last to the page.

* M refers to the methods of work, the numeral to the number of the method referred to.

two nuclei in the same cell is in proportion to the development. We have seen that in the cells of Purkinge the existence of two nuclei is the rule, and yet it is scarcely probable that this arrangement is connected with the complete fusion of two cells at first distinct."

In 1861 Weismann presented a paper (6.41-61) in which he says that cardiac muscle is distinguished from ordinary striated muscle in that its sarcolemma, if it has any, is so delicate that it has not yet been demonstrated; that the fibers are more slender; that they have a granular appearance; and that they have a multitude of anastomoses. He also mentions the presence of two nuclei in some cases, states that in cross section the fibers are irregular in size and shape, and calls attention to what he styles leaf-like ribs on the surface of the cardiac cells in frog and rabbit (6.44,55).

In his investigations on the human cardiac tissue (6.55) he noted tran-striæ in the cells of an embryo of four months, and stated that the striæ first appear in the substance at the periphery of the cell, after which the zone of substance next within begins to assume a similiar appearance, and so on until the entire substance of the cell, except the nucleus has become striated; also that in the embryo the fibers were made up of cells set in like tile work. They were without a membrane and were held together by tissue cement. These cells, however, which in the embryo were independent and simply placed together, became blended into a multitude of fibers (6.56). In another example, that of a child born breathing, at six months the longi-striæ were also visible. The size of the cells in this case was greater than in the one last mentioned. In both clear nuclei were visible.

In regard to the adult human cardiac muscle he says: "Fragments obtained by the use of potassium hydrate, often show the original composition of cells, for crooked lines pass over them, and, corresponding to these, the ever present nuclei are distributed." In another place he says: "The

originally separate cells are recognizable in the adult only by slight indications."

In his *Traité Technique D'Histologie* (5.540) Ranvier says: "In subjecting to the action of potassium hydrate fragments of the heart of different mammals, cells of much larger size than those in the frog, and of different form, may be isolated. They have a smooth cylindrical surface which corresponds to that of a muscular fiber. The two bases of the cylinders are sinuous and are joined to one another by a layer of cell cement, so that they can be recognized upon those parts of the preparation where the cells have not been completely isolated."

With reference to the development he says (4.296): "The initial embryonic cell is separated into two parts, the one formed of nuclei, and sown with protoplasmic granules is the protoplasmic zone; the other the substance of which forms in its definite arrangement the striated contractile substance, is the muscular zone of the element. This separation of the protoplasmic substance into two distinct regions of the same cell exists in the early stages of the development of all striated muscle, in the higher vertebrates as well as in the amphibia and birds; but it is transitory and purely embryonic. In the heart of certain animals, on the contrary, especially ruminants, this same form, known as the fibers of Purkinge, is persistent in the adult state and serves to trace the union between ordinary and cardiac muscle."

Comparison of the Adult Structure in Man and Certain Other Mammals.

(The scale lines with the figures are 100dths. mm. The full black lines represent the cell-cement.)

In making these comparisons I will begin with man as a standard.

As was stated by Kölliker and also by Weismann, the human cardiac muscle has no sarcolemma, the fibers are more slender, there is a granular appearance, and the fibers

possess multitudinous anastomoses of varying size and shape. Even Kölliker recognized that the cell is the histological element of the cardiac muscle, but he held that in the adult they were fused with one another. Weismann maintained the same thing, but in another place, as spoken of above, he mentioned the lines of connecting cement, yet as if they were merely traces of original cell divisions. In fact he says: "The original cells are recognizable only by signs." On the other hand Ranvier seems to imply that he had no difficulty in separating *any* of these cells. In my work on the adult human cardiac muscle I have found the truth to lie between these two extremes, some cells readily separating, others not separating but showing the lines of cement, and yet others in which the lines, if present, were not visible, although one would have expected them to appear Fig. I., 3. I found the above to be true in each of the three specimens of human cardiac muscle examined. Frequently fatty granules were found in the cell substance, as mentioned by Kölliker, and which are without doubt identical with the "granular appearance" noted by Weismann. The tran-striæ were very distinct, but the the longi-striæ were not noticeable until acted upon by some reagent as chromic or nitric acid (M. 3, 4). Each cell had one nucleus in the interior of its substance. In but a single instance did I observe two nuclei in the same human cardiac cell (Fig. I., 4.)

The human cardiac cells are, as compared with the size of the body, smaller than in any other animal examined (see table at the end of heading). Owing to their position within the substance of the cell, the nuclei frequently did not appear until acted upon by a reagent.

The lowest form I have been able to examine is the oppossum, and in several particulars its cells are strikingly different from those of man. In all the cells where nuclei were observed they were two in number (Fig. II., 2), and they were larger compared with the size of the cell than in any other animal examined. The fibers also presented a very

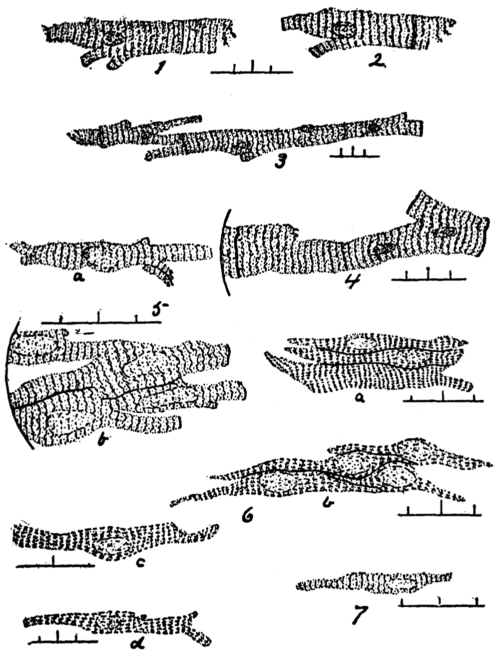

FIG. I. MAN.

Fig. I.—*Man.* 1, 2 and 3, are from the heart of a man 33 years old. All these show the branchings as seen in man, and that many cell divisions were clearly visible. 3 shows also that these cell divisions are not always visible where one would expect to find them. These were isolated by *Method* 1. 1 and 2 remained in the liquid ½ hour; 3, 1 hour. 4 is from the heart of an old man. It shows that, even in old age, the cell divisions are present. The two nuclei here present in one cell is the only case noticed by me in human cardiac muscle. This was obtained by *Method 2.* 5 is a child at term. It shows that the branches are numerous at this stage. The nuclei are larger than in the adult, but smaller than at six months (see table on p. 295). This was obtained by *Method 1.* 6 is of an embryo at six months. It shows the tran- and longi-striæ about equally prominent. Here the nuclei are at their maximum size (see table on p. 295). This was obtained by *Method 1.* 7 is of an embryo at four months. It shows only tran-striation. The nucleus has not attained its maximum size. There are no branches. This was obtained by *Method 1.*

striking appearance, having frequent, but not regularly placed, conctrictions,—an appearance as if they had been tied about with cords, and which gave them a scalloped outline (Fig. II.)

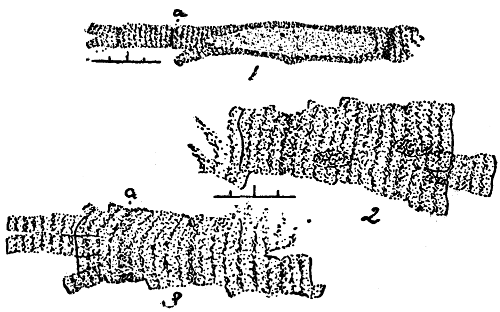

FIG. II. OPPOSSUM.

Fig. II.—*Oppossum.* This was an adult animal. 1 was prepared by *Method 3*, and mounted in carmine-glycerine. 2 and 3 were prepared by *Method 1*. 3 shows something of the numerous branches found in the animal's heart fibers. 1 shows an optical section of a fiber, which, by focussing, was seen to possess striæ above and below, showing that it was a cylinder of striated substance and granular protoplasm within. 1 and 3 show constrictions which were very abundant on the fibers.

Then the number of anastomosing branches is greater than in the human cordiac muscle. But what is even more striking is an appearance as if the fibers were hollow cylinders, and in this respect they present a persistent embryonic condition comparable with that found in the cells of Purkinge. It needs, however, more careful study than the time now at my disposal will permit, in order to reach the full meaning of this appearance. Of course until that has been done any conclusions would be premature.

Among the rodents, in the squirrel and mouse, two nuclei are nearly always present in the cardiac cell, but they are not so large relatively as in the oppossum. The branchings are still present although not so strikingly abundant. (Figs. III., IV.)

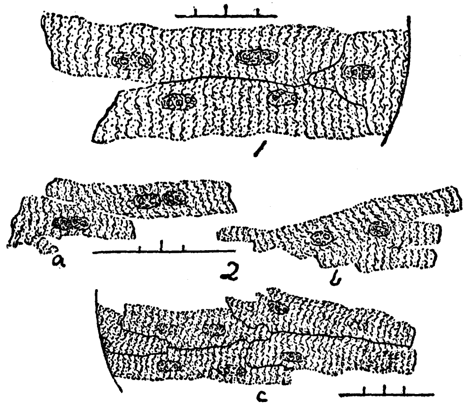

FIG. III. SQUIRREL.

Fig. III.—*Squirrel.* All these drawings were made from potassium preparations. 1 was in the liquid 48 hours and all of 2 from 12 to 16 hours. Two nuclei are quite constant. 2, a, shows the nuclei in a very interesting position (see p. 288). 2, c, shows the relation of neighboring cells.

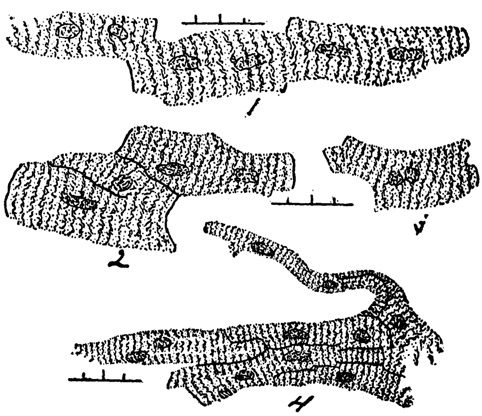

FIG. IV. MOUSE.

Fig. IV.—*Mouse.* These were all drawn from potassium preparations. Here, as in the squirrel, the two nuclei are quite constant. Compare 3 with Plate VI, 2, a. and see p. 288. 4 shows a very interesting relation of cells.

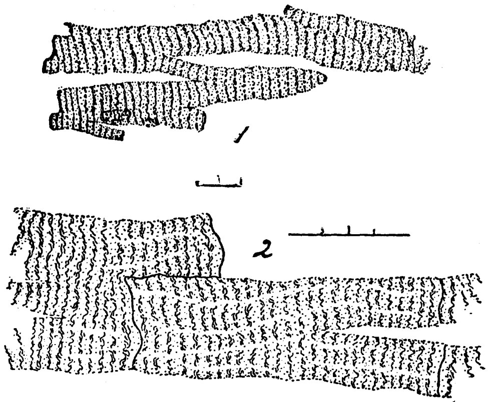

FIG. V. COW.

Fig. V.—*Cow.* The preparations for this figure were from the heart of an *old* cow. 1 was prepared by *Method 1*, 2, by *Method 4*. The cells are very large (see table on p. 292). The nuclei, in many cases, were not distinct, and the cell divisions were not so noticeable as in the cat or dog. 1 shows peculiar cell endings which resemble those found in amphibians and reptiles.

Among the ruminants, as for example the ox, the cell divisions were not so distinct as in the lower forms, or as in some of the higher ones (compare Fig. III., IV. and V.) The animal whose heart was examined was old, and perhaps this may in part account for this peculiarity. I found one condition in this heart which I have not found anywhere else. There were two cells, each of which had an ovately conical end, in place of the usual either abrupt or branching end. This recalls the persistent condition of the cardiac cell in amphibians and reptiles. In the ox the cells were absolutely, but not relatively, larger than in any of the other animals examined (Fig. V., and table at close of this heading).

Among the carnivora, as for instance in the dog, the cells were quite distinct and easily separable in potassium hydrate. Two nuclei still seem to be the rule. In the cat the structure is very similiar to that of the dog, except that there are more branches (Figs. VI., VII).

Among primates, in the monkey, the cardiac cells are also very frequently doubly nucleated, and they are far more easily separable in potassium hydrate than is the case with man. The nuclei, relatively to the size of the cell, are smaller than in man, although the cells themselves are proportionally much larger, being absolutely about one-third larger, while the body is only about one-twentieth of that of man.

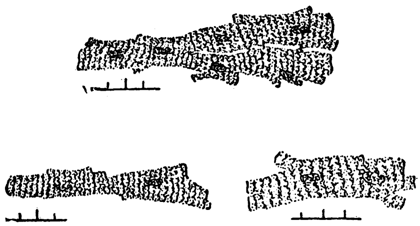

FIG. VI. CAT.

Fig. VI.—*Cat.* These are from potassium preparations which had been in the liquid about 1 hour. They show an increase in branchings over those of monkey. The presence of two nuclei is also more frequent. The animal was adult.

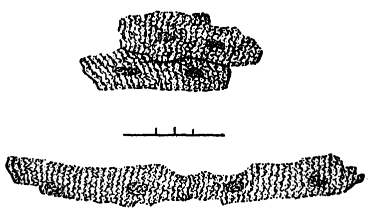

FIG. VII. DOG.

Fig. VII.—*Dog.* Potassium was used in making the preparations from which these drawings were taken. It was an adult terrier dog. It shows a structure very similiar to that of the cat, except that there were not so many branchings.

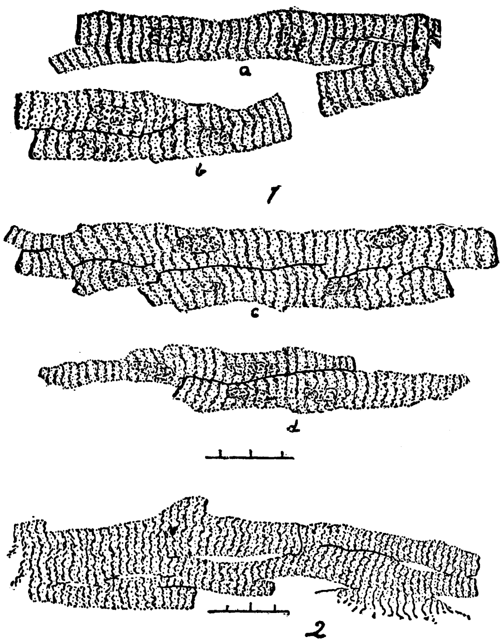

FIG. VIII.

FIG. VIII.——These drawings are from preparations of the heart of an adult monkey. Those included under 1 were made from preparations isolated by means of *Method* 3, those under 2 from preparations isolated by means of *Method* 3.

The drawings show the enormous increase in the size of the cell as compared with man (see table below), and the great frequency with which two nuclei occur. The absence of nuclei in 2 is due to the method of preparation. 1, c, is a good illustration of the manner in which neighboring fibers anastomose.

A Table of Comparisons of the Average Adult Size of Cells and Nuclei.

	CELL.		NUCLEUS.		
NAME.	AVERAGE LENGTH.	NUMBER AVERAGED	LONG DIAMETER.	SHORT DIAMETER.	NUMBER AVERAGED
Man	69.9μ.	5	11.4μ.	5.2μ.	9
Monkey	99 6μ.	5	22.9μ.	4.8μ.	14
Cat	70.6μ.	5	9.6μ.	4.2μ.	6
Dog........	87.9μ.	4	10.2μ.	4.8μ.	8
Ox	114.5μ.	5	14.5μ.	6.7μ.	2
Squirrel	90.4μ.	5	10.9μ.	5.4μ.	10
Mouse	69.4μ.	5	11.1μ.	5.5μ.	10
Oppossum..	106.8μ.	4	21.4μ.	10.8μ.	5

NOTE.—The object of the above table, as well as the one at the close of the following heading, is to give a truer comparison by eliminating as much as possible the individual variations. It seemed best

not to make an average of the cell diameter, as in this case the individual variation was such that no uniform place of measurement could be obtained ; and therefore any results thus obtained would be misleading rather than helpful.

Comparison of the Structure in Man at Different Stages of Development.

I have not succeeded in obtaining any very young human embryos. But Foster and Balfour say (1.269) that in the early stages of development the cells are spherical or oblong and gradually become fusiform.

The youngest specimen examined by me was that of an embryo at about four months. In this the cells were about forty microns in length and fusiform, but with some irregularity of outline (Fig. I., 7). There were tran-striæ quite distinctly visible ; but no longi-striæ were observed. In this Weismann concurs, as was above mentioned (p. 284). The nuclei were more transparent than the surrounding substance.

The next example studied was that of an embryo at about six months. In this the cells were of greater size, and the nuclei were relatively larger. They were so large as to cause the surrounding part of the cell to bulge outward ; and the overlapping ends of neighboring cells were indented (Fig. I., 6, b, c).

Here in many cases the tapering ends of the cells showed enlargements and, in some cases indications of the adult anastomosing condition of the cells. Here also longi-striæ had become visible, and it was difficult to determine whether these or the tran-striæ were the more prominent. In all the preparations of embryonic cardiac muscle examined the cell outlines were very distinct (Fig. I., 6-7), and there was not the slightest indication of fusion.

The next specimen examined was that of a child at term. In this the nuclei were still large compared with those of the adult, but smaller than those in the preceding specimen ; the branchings, indications of which were seen in the

embryo at six months, had been effected (Fig. I., 5); tran-striæ had become more prominent again; the length of the cells had increased to between fifty and sixty microns; and the nuclei still appeared more transparent than the surrounding substance.

I have, as yet, been unable to obtain any specimens of adolescent human cardiac muscle, but judging from the difference which exists, between the child at term and the adult, these stages must display many interesting and instructive features.

In the adult the size of the cell had increased while that of the nucleus had decreased and no longer appeared as a clearer portion of the substance, but as a more dense one. The branchings had become more numerous; and whether the cells had amalgamated or not, they were less readily separable in the reagents at present in use. In some cases the lines of cement did not appear where one would have expected to find them (Fig. I., 3). I have not, however, found, as regards the human cardiac muscle, that the cells are less readily separable in the old than in the middle aged. There seemed, also, to be as many cell divisions visible. The only case of a doubly nucleated cell which I have observed in man, was found in the cardiac muscle of an elderly person.

Weismann says (6.55): "The mammalian heart shows first the structure of the mollusk, then of the fish as it passes through the different stages of development." It would also seem natural that the human cardiac cell in the course of its development, should show the structure which is found in the adult lower forms, yet in none of the embryos examined did I find more than one nucleus in a cell. It would, therefore, seem that these double nuclei, so common in most mammals, are the result of a later segmentation of the nucleus or of the amalgamation of cells — probably the former. One fact which would seem to indicate this, in addition to those presented in the historical sketch, is that

if the cell, or if you prefer it, the segment, which contains two nuclei was the representative of two cells, it would make these resulting cells too small to maintain the ratio with those which have but one nucleus in the earlier stages of development. Then, there are some cases in which the nuclei are not entirely separate or are very close together, and smaller than usual, which would make it appear unlikely that these nuclei represent two originally distinct cells (Figs. III., 2, a; IV., 3).

A Table of Comparison of the Average Size at different Stages of Development.

HUMAN EMBRYO.	CELL.		NUCLEUS.		
AGE.	AVERAGE LENGTH.	NUMBER AVERAGED	LONG DIAMETER	SHORT DIAMETER	NUMBER AVERAGED
Four months.........	42. μ	1	7.8μ	2.6μ	1
Six months..........	64.7μ	6	12.9μ	6.8μ	5
Nine months.........	64.3μ	5	12.4μ	5.6μ	7
Adult	69.9μ	5	11.4μ	5.2μ	9

NOTE.—The apparent absence of growth, from the sixth to the ninth month, may be due to the fact that during this time the cells are expanding in breadth rather than length. (See also note at close of last heading.)

METHODS OF WORK.

I. *Methods of Preparation.*

1. The Potassium Hydrate Method: This was introduced by Moelschott and has been in use ever since.

In a 35 to 40 per cent. (35 to 40 grms. K O H and 65 to 60 c.c. H_2 O) aqueous solution of caustic potash a fragment of muscle is placed for from fifteen minutes to as many hours. A weaker solution dissolves the cells instead of separating them.

It is much more effective if the blood is first soaked out of the portion of muscle to be used, since if this is not done

the preparation will appear cloudy and the details can not be clearly seen. This is the most effective of any of the methods which I have tried for dissolving the cell cement and so for separating the cells. It does not bring out the longi-striæ, but it does make the nuclei very distinct if it acts for an hour or more.

Permanent preparations may be made, where this solution has been used, as follows: Add to the edge of the cover enough potassium acetate to replace the alkali, then stain; add glycerine and seal as usual.

2. Frozen Sections: This method is, in some respects, better than the preceding, as it leaves the tissue in a more nearly natural condition. But it necessitates having a freezing microtome, and so, in some cases, might not be available.

It consists in taking a small piece of muscle, say a cubic centimeter (not more, as the sections can thus be made thinner than if a larger piece were taken), which is then frozen by either spray or other means as the microtome may require. Care should be taken not to freeze the tissue too hard, since just as much difficulty is experienced in making good sections when it is frozen too hard as when not frozen hard enough. The proper degree of hardness can be easily determined after a little practice. After the sections have been made it is best to float them out in water. They may then be stained and mounted in the ordinary manner.

3. The Nitric Acid Method: This method was devised by Reichart in 1849. It consists in macerating the tissue in weak nitric acid (concentrated $H N O_3$, 23 parts, $H_2 O$, 77 parts) for about three days. The exact time can be determined only by examination, as it varies in different cases. When it is sufficiently macerated, the acid is removed by soaking in water for several hours, after which it may be teased out with needles and stained. This method brings out the longi-striæ very nicely.

4. Chromic Acid Method: This method is recommended by Ranvier (5. 54) as the best method to demonstrate the cell-cement. A piece of muscle is placed for twelve hours or more in a one-tenth per cent solution of chromic acid; then after washing with water it may be teased out with needles, stained in picro-carmine, and mounted in formic acid one part, to glycerine ninety-nine parts. This brings out both systems of striæ finely as well as the cell cement.

II. *Methods of Staining.*

The stain needed is one to bring out the nuclei and the cell-cement. For the fresh sections, obtained with the freezing microtome, I found that ammonia-carmine gave excellent results, but did not acertain whether it is applicable to preparations obtained by means of reagents. With nitric acid preparations, a mixture of glycerine 90 c. c. and alum-carmine 10 c. c. proved very satisfactory, both as a stain and as a mounting medium.

III. *Methods of Observation.*

In this investigation, powers ranging from 320 to 1000 diameters were used, but usually about 500.

IV. *Methods of Drawing.*

In making the drawings I used constantly the Abbe' camera lucida. This has the great advantage of permitting the microscope to remain in an upright position, so that there is no danger of the object moving out of the field of view before the drawing is completed. The measurements were made by means of the stage micrometer, thus avoiding any error from change in the length of the tube, or from any mistake in calculating the power. It simply magnifies the micrometer just as much as, and no more than, it does the object. The only chance for error is in drawing the lines of the micrometer on the paper. By drawing a large number of these lines on the paper and taking their average, the error is reduced to a minimum.

The permanent drawings were made, in all cases, from those taken under the camera. This was accomplished by tracing, so that the amount of variation, aside from any defect in the use of the camera, could not be more than twice the width of the pencil's point. The filling in of the striæ was freehand and arbitrary. It only aims to produce, as nearly as may be, an impression upon the eye similar to the one made by the object under the microscope. Perhaps this is most nearly realized by holding the drawing at about two feet from the eye. (The drawings were reduced one-half, Ed.)

BIBLIOGRAPHY.

1. Foster, M. and Balfour, F. M.
Elements of Embryology. 2d Edition.

2. Leuwenhoek.
Experimenta et Contemplationes. Op. Om. Lugd. Bat. Tome I., p. 409. 1722.

3. Kölliker, A.
Handbuch des Gewebe lehre des Menchen. One volume, Leipzig. 1867.

4. Ranvier, L.
Leçons D'Anatomia Général. One volume. Paris. 1880.

5. Ranvier, L.
Traité Technique D'Histologie. One volume. Paris. 1875.

6. Weismann, A.
Ueber die Musculatur des Herzensbeim Menschem und in der Thierreihe. Archiv. für Anatomie. 1861.

COMPLETE BIBLIOGRAPHY.

1. Quain.
Elements of Anatomy. Ninth edition, two volumes. London. 1882. References on heart. Vol. II., p. 501.

2. Index Catalogue of the Library of the Surgeon General's Office, U. S. A., Vol. V., p. 956, gives a complete list of literature on the heart, contained in periodicals, up to June, 1884.